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Running head: Metabolic rate and feed efficiency in fish

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[1]This publication was made possible through support provided by CIRAD and the CGIAR Research Program on Fish Agrifood Systems (FISH) and the International Fund for Agricultural Development (IFAD). The authors are grateful to the Ifremer Experimental Aquaculture Research Station staff and facilities and to H2020 AQUAEXCEL2020 (No. 652831).

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ABSTRACT: Feed efficiency (FE) is the amount of body weight gain for a given feed intake. Improving FE through selective breeding is key for sustainable finfish aquaculture but its evaluation at individual level is technically challenging. We therefore investigated whether individual routine metabolic rate (RMR) was a predictor of individual FE in the European sea bass *Dicentrarchus labrax*, a major species in European mariculture. The European sea bass has three genetically distinct populations across its geographical range, namely Atlantic Ocean (AT), West Mediterranean (WM) and East Mediterranean (EM). We compared FE and RMR of fish from these three populations at 18°C or 24°C. We held 200 fish (62 AT, 66 WM and 72 EM) in individual aquaria and fed them from *ad libitum* down to fasting. Feed efficiency was assessed for an *ad libitum* feeding rate and for a fixed restricted ration (1% of metabolic body weight.day^{-1}, with metabolic body weight = body weight$^{0.8}$). After being refed 12 weeks in a common tank, individual RMR was measured over 36h by intermittent flow respirometry. There was a significant effect of temperature whereby fish at 18°C had greater mean FE ($P < 0.05$) and lower RMR ($P < 0.001$). There was also a significant effect of population, where AT fish had lower FE ($P < 0.05$) and greater RMR ($P < 0.001$) than WM and EM, at both temperatures. Despite these differences in temperature and population means, individual FE and RMR were not significantly correlated ($P > 0.05$). Therefore, although the

results provide evidence of an association between metabolic rate and FE, RMR was not a predictor of individual FE, for reasons that require further investigation.

Key words: Aquaculture, genetic populations, individual rearing, oxygen consumption, respirometry**.**

LIST OF ABBREVIATIONS

AT, Atlantic

BW, body weight

BWG, body weight gain

BWG_1%, body weight gain for a restricted feed intake set to 1% of metabolic body weight.day^{-1}

dph, days post hatch

EM, East Mediterranean

FE, feed efficiency

FI, feed intake

MBW, metabolic body weight

MO_2, measurements of oxygen uptake

resBWG, residual body weight gain

RMR, routine metabolic rate

SMR, standard metabolic rate

WM, West Mediterranean

INTRODUCTION

Finfish aquaculture represents a promising source of sustainable animal protein for growing human populations globally (Godfray et al., 2010; Froehlich et al., 2018; FAO, 2018). A major factor in aquaculture sustainability is, however, feeding the fishes: it is the single greatest cost (Goddard, 1996) and a major determinant of environmental impact (Besson et al., 2016a). Feed efficiency (FE) is the ratio between fish body weight gain (BWG) and feed intake (FI); selective breeding to improve FE is a promising means to make aquaculture more sustainable (Besson et al., 2014; Besson et al., 2016a). Farmed fishes are, however, reared in large groups, where individual FI is technically very challenging to measure (Kause et al., 2006; Grima et al., 2008). An alternative is to rear individuals singly (Silverstein, 2006; Martins et al., 2011; Besson et al., 2019; Rodde et al., 2020), a technique that is widely used on terrestrial livestock (Luiting and Urff, 1991; de Haer and Merks, 1992; Nguyen et al., 2005; Drouilhet et al., 2016). This method has already been applied to European sea bass *Dicentrarchus labrax*, a major species for finfish aquaculture in Europe (Besson et al., 2019; Rodde et al., 2020). It involves holding hundreds of animals for several weeks, provided with individual daily rations, with all uneaten feed carefully collected and quantified each day (Besson et al., 2019; Rodde et al., 2020). In the European sea bass it returned reliable estimates of individual FE, to the extent that individual FE in aquaria was clearly linked to the subsequent FE of groups, groups being composed of fish graded for individual FE (Besson et al., 2019). The method is, however, extremely time-consuming and laborious, which impedes its application on a large scale for selective breeding programs in aquaculture.

It is, therefore, useful to search for indirect selection criteria of individual FE, that are accurate predictors and can be measured more easily and rapidly. Bioenergetics is promising because it is assumed that energy intake from feed in fishes is allocated among several

processes, most notably maintenance metabolism, activity and growth (Warren and Davis, 1967; Bureau et al., 2003). It is conceivable that, for a given feed intake, the most efficient individuals will be those that allocate the least energy to maintenance and activity, and the most to growth. In terrestrial livestock such as cattle, sheep and poultry, there is clear evidence of a negative correlation between individual metabolic rate and FE (Luiting et al., 1991; Nkrumah et al., 2006; Arndt et al., 2015; Chaves et al., 2015; Paganoni et al., 2017). Although there is some evidence of a link between metabolic rate and FE in groups of fishes (Kinghorn, 1983; Zeng et al., 2017), this remains to be demonstrated at an individual level.

This study investigated whether individual metabolic rate, measured indirectly as rates of oxygen uptake, was a predictor of individual FE in the European sea bass. In this species, individual metabolic rate is negatively correlated with mass loss during fasting (Killen et al., 2011; McKenzie et al., 2014), indicating a link between metabolism and non-growth energy requirements, which could also relate to individual bioenergetics when feeding. Measuring individual oxygen consumption by respirometry on fasted (post-prandial) fish is technically quite simple and takes less than 48h per animal (McKenzie et al., 2014).

Three genetically distinct populations of European sea bass have been identified across its natural geographical range: Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM; Guinand et al., 2017). These populations started to diverge 300,000 years ago (Duranton et al., 2018; Duranton et al., 2020) in environments whose temperatures differed along a North-West to South-East temperature gradient (Lindgren and Håkanson, 2011). European sea bass farming is by coastal cage mariculture so it is valuable to understand whether the populations may differ in their bioenergetics at different water temperatures, with potential implications for selection programs in different areas of Europe.

Rodde et al. (2020) measured individual BWG and FI, over a range of feeding rates from *ad libitum* down to fasting, in 200 European sea bass from AT, WM and EM

populations, at two temperatures, 18 and 24°C. These two temperatures are representative of the coolest and warmest sites where European sea bass is reared across Europe (Vandeputte et al., 2014), and correspond to the average and optimal temperatures, respectively, that European sea bass undergoes when farmed in sea cages in the West Mediterranean (Person-Le Ruyet et al., 2004; Besson et al., 2016b). In the present study, we evaluated the metabolic rate of these individuals, by respirometry, at their acclimation temperatures. We then compared this to FE, of individuals and temperature by population combinations, considering when they were fed either *ad libitum* (their individual maximum energy intake) or with a fixed restricted ration of 1% individual body mass per day. Fish with greater metabolic rates may compensate by consuming more dietary energy than others when fed *ad libitum* but would be unable to do so on a fixed ration. That is, metabolic rate might be more closely related to FE when measured for a restricted ration compared to *ad libitum*. This study is, to our knowledge, the first to assess whether individual metabolic rate is a predictor of individual FE in farmed fish.

MATERIALS AND METHODS

Ethical Approval

Experimental procedures were approved by C2EA−36 ("Comité d'éthique en expérimentation animale Languedoc-Roussillon") under authorisations APAFiS n° 2018032109435819 and n° 2018100910598940.

Animals

Complete details of how the fish were produced, reared and evaluated for their individual FE are provided in Rodde et al. (2020). Briefly, 200 European sea bass from the three populations (62 AT, 66 WM and 72 EM) were produced on a single day by controlled breeding at the Ifremer Experimental Aquaculture Research Station (Palavas-les-Flots,

France). Fish were reared at a mean (± SD) temperature of 16.2 ± 0.9°C from 0 to 97 days post hatch (dph), then of 24.4 ± 0.8°C from 98 to 152 dph, and finally of 21.1 ± 1.0°C from 153 to 221 dph.

Measurement of Individual Bodyweight Gain and Feed Intake

When fish reached 221 dph and a mean mass of 23.4 ± 8.4g, they were transferred to an individual rearing system to estimate their individual FE. The individual rearing system consisted of two independent recirculating water systems, respectively set to 18 and 24°C, each comprising 100 individual aquaria (vol. 10L), a sand filter, a biological filter and a ultraviolet filter. Feed, for the whole rearing period was a commercial diet called "Neo Start 3" (Le Gouessant Aquaculture, Lamballe, France). This diet was comprised of 47% of crude protein, 18% of crude fat, 1.5% of crude fibre, 8% of ash, 1% of phosphorus; digestible energy content was 19 $MJ.kg^{-1}$, digestible protein/digestible energy ratio was 23 $g.MJ^{-1}$. Feed was supplied once a day in the morning (9 a.m.) by automatic feeders set on the cover of each aquarium. The 200 aquaria were arranged on racks in the same room where they were shielded from visual disturbance, except once a day when uneaten feed was collected and quantified for each fish. Individuals could see their conspecifics in adjacent aquaria. Each aquarium received a constant supply of biofiltered aerated water at the appropriate temperature: water renewal was 300% per hour and mean oxygen saturation was 114.1% (8.64 mg $O_2.L^{-1}$) and 107.1% (7.28 mg $O_2.L^{-1}$) at 18 and 24°C, respectively. Mean water salinity was 37.2‰ and 37.4‰ at 18 and 24°C, respectively, and mean water pH was 8.3 in both cases. Photoperiod was artificial: 12h light/12h dark (Rodde et al., 2020).

To acclimate fish to individual rearing, they were first held in groups of five per aquarium from 221 to 235 dph, then they were isolated and held, alone, for a further two weeks of acclimation. Once so acclimated, fish were fed incremental rations until their individual *ad libitum* feeding rate was identified, over three successive periods of seven days.

Once individual *ad libitum* feeding rate was identified, fish were exposed to six sequential reductions in ration, from *ad libitum* (100%), to 80%, 60%, 40%, 20% and then 0% (fasting). Fish were fed 100% *ad libitum* for 22 days, then for 10 to 11 days at the other steps; fish remained in isolation for 123 days (Rodde et al., 2020). Individual BWG and FI were measured at each step, to calculate fish FE *ad libitum* and at a ration of 1% individual body mass per day, see below.

After these trials, fish were grouped into two common tanks (vol. 1000L) supplied with biofiltered water at either 18°C or 24°C and fed *ad libitum* for 12 weeks. This period ensured that fish were in a stable nutritional state and that physiology and behaviour were not directly influenced by the feed deprivation or any stress linked to individual rearing (Dupont-Prinet et al., 2010; Rubio et al., 2010; McKenzie et al., 2014).

Metabolic Rate by Respirometry

One week before respirometry, fish were distributed among three holding tanks per temperature (vol. 1000L) supplied with biofiltered water. Single tanks were fasted for 24h sequentially, and then, in the afternoon, up to 32 fish were netted, identified by a passive integrated transponder tag and weighed. The three tanks meant that fish were not disturbed by netting more than once every 72h. They were then placed into individual semi-transparent respirometry chambers (volume either 1.8 or 3.0L) according to their size, such that they were free to move easily, and left for 12h (overnight) to recover from handling. There were 32 respirometry chambers, placed submerged on two polyvinyl chloride trays (100 x 200 x 28 cm) supplied with aerated biofiltered seawater at either 18°C or 24°C. The trays were shielded behind opaque black plastic to avoid visual disturbance, with the fish in dim light at an artificial photoperiod of 12h light/12h dark. In that system, individuals will have been aware of their conspecifics in adjacent chambers.

187 Measurements of oxygen uptake (MO_2) were made by intermittent stopped-flow
188 respirometry (Steffensen, 1989) as described in McKenzie et al. (2014), but with a 15 minute
189 cycle comprising eight minutes stopped flow and seven minutes flushing with aerated water.
190 Water oxygen levels in the chambers were measured and recorded every ten seconds by
191 optodes (Firesting OXROB10 oxygen sensors, www.pyroscience.com) and associated oxygen
192 meter (Firesting FSO2-O4) and software (Pyro Oxygen Logger). During stopped flow,
193 oxygen saturation in the chambers declined due to consumption by the fish, MO_2 was
194 calculated as mg $O_2.kg^{-1}.h^{-1}$ considering the volume of the chamber and the solubility of
195 oxygen in seawater at 18 or 24°C and a salinity of 37‰ (Steffensen, 1989; Dupont-Prinet et
196 al., 2010). After the 12h recovery from handling, measurements were collected for 24h on
197 undisturbed fish. Upon removal of a batch from their chambers, background oxygen
198 consumption due to bacterial respiration was measured over 20 min in the sealed chambers
199 (Svendsen et al., 2016), this represented about 2% of the total oxygen consumption by the
200 fish, so no correction was applied.

201 ***Data Analysis and Statistics***

202 All statistical analyses were performed with R software (R Core Team, 2018). The
203 normality of residuals was checked using the quantile-quantile method (comparing residuals
204 quantiles with theoretical normal quantiles). The homoscedasticity and independence of the
205 residuals were checked by comparing the residuals with the fitted values from the models.
206 Linear mixed models and tests associated to these models were performed using R packages
207 "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017).

208 ***Feed efficiency: Relationship between BWG and FI.*** The measures of BWG and FI
209 were standardized to (divided by) metabolic body weight (MBW) instead of body weight
210 (BW), based upon a mass exponent for metabolic rate of 0.8 in European sea bass ($MBW = BW^{0.8}$; Lemarié et al., 1992; Lupatsch et al., 2003; Rodde et al., 2020). Both BWG and FI

were then expressed as a percentage of MBW. The following repeated measures linear mixed model was used to estimate individual FE as residual BWG (resBWG) when fish were fed *ad libitum*:

$$BWG_{ij} = intercept + slope * FI_{ij} + A_j + B_j * FI_{ij} + resBWG_{ij}$$

where BWG_{ij} and FI_{ij} are, respectively, the BWG and FI at step i (i between 1 for *ad libitum* and 6 for fasting) for animal j, A_j and B_j are the random effects of the animal j, respectively, associated with intercept and slope, with $A_j \sim N(0;\sigma^2_a)$, with $B_j \sim N(0;\sigma^2_b)$ and $resBWG_{ij}$ the residual of the model ($resBWG_{ij} \sim N(0;\sigma_e^2)$). From a biological point of view, the greater the resBWG, then the more efficient the individual. The model was calibrated on all the data from the six different feeding rates to increase its robustness, although only $resBWG_{1j}$ (resBWG *ad libitum*) were to be extracted from the model for further analyses.

Then, the following linear model was used for each fish to estimate the intercept and slope of the linear relationship between its BWG and FI:

$$BWG_i = intercept + slope * FI_i + \varepsilon_i$$

where BWG_i and FI_i are respectively the BWG and FI at step i (i between 1 for *ad libitum* and 6 for fasting) for each fish, and ε_i the residual ($\varepsilon_{ij} \sim N(0;\sigma_e^2)$). The intercept and slope of this relationship permitted to predict for each fish its BWG (in % of MBW) for a restricted FI set to 1% of MBW.day^{-1}, abbreviated as BWG_1%, with BWG_1% = intercept + slope*1.

Calculation of Metabolic Rates. Rates of oxygen uptake were corrected to (divided by) MBW, as per the measures of BWG and FI. Routine metabolic rate (RMR), defined as the metabolic rate of post-absorptive, undisturbed, resting animals at their acclimation temperature, which also includes the costs of random activity and the maintenance of posture and equilibrium (Killen et al., 2011), was taken as the mean rate of MO_2 over 24h. Standard metabolic rate (SMR), defined as the minimal energetic cost of living for an ectotherm at their

acclimation temperature, was estimated as the 0.25 quantile of MO_2 values over the 24h period (Chabot et al., 2016).

Variation in Phenotypic Traits between Temperatures and among Populations. The following linear model was used to determine the variation of each trait at temperature and population levels:

$$Y_{ijk} = \mu + T_i + P_j + TP_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the phenotypic trait considered at temperature i (18°C or 24°C), for genetic population j (AT, WM or EM) and animal k; μ is the general mean, T is the fixed effect of temperature, P is the fixed effect of population, TP the interaction of these two effects, and ε_{ijk} the residuals ($\varepsilon_{ijk} \sim N(0;\sigma_e^2)$). Regarding the three populations, their pairwise differences were further explored using Tukey post-hoc test.

Link between Feed Efficiency and Metabolic Rate among Individuals. Pearson's correlation coefficient was estimated between traits of FE and metabolic rate, for each single temperature by population combination.

RESULTS

Among the 200 fish held, six died before the phenotyping period because they jumped out of their aquarium (five AT at 18°C and one AT at 24°C). Of the 194 fish that were phenotyped in the aquaria, 46 failed to have a positive growth rate when fed *ad libitum*: seven AT out of 28, five WM out of 34 and six EM out of 37 at 18°C; 11 AT out of 28, 13 WM out of 32 and four EM out of 35 at 24°C. The resBWG and metabolic rate were not estimated for fish that lost weight *ad libitum*. For ethical reasons, if a fish had lost weight when fed *ad libitum*, or to 80%, 60% or 40% of *ad libitum*, the next step was fasting, with the fish then removed from the experiment. Moreover, five fish (two AT at 18°C, two AT at 24°C and one WM at 24°C) did not eat at all when fed *ad libitum* (< 1% of their BW over the 22 days) and were therefore removed from the experiment, without any fasting step. The individual linear

model to calculate BWG_1% was only applied to the 107 fish that were successively phenotyped for at least 100%, 80%, 60% and 0% of *ad libitum* feeding rate, to have at least four data to calibrate the individual linear model (Rodde et al., 2020).

Mean mass at the end of the individual rearing period was 31.3 ± 12.0g at 18°C and 35.4 ± 11.4g at 24 °C. After 12 weeks of *ad libitum* refeeding into common tanks, a threefold and fourfold increase in body mass was observed at 18 and 24°C, respectively: when respirometry was performed, the mean mass was 99.0 ± 29.7g at 18°C and 146.2 ± 40.4g at 24°C. Among the 148 fish that were successfully phenotyped *ad libitum*, 120 fish (18 AT, 19 WM and 25 EM at 18°C and 14 AT, 17 WM and 27 EM at 24°C) had their metabolic rate successfully estimated. Moreover, among the 107 fish that had their BWG_1% estimated, 92 fish (14 AT, 13 WM and 22 EM at 18°C and 7 AT, 12 WM and 24 EM at 24°C) had their metabolic rate successfully estimated.

Variation in Phenotypic Traits between Temperatures and among Populations

Firstly, resBWG (*ad libitum* feeding rate) as well as BWG_1% were significantly different between temperatures ($F_{1,126} = 4.75$, $P = 3.11\times10^{-2}$ and $F_{1,103} = 8.88$, $P = 3.59\times10^{-3}$, respectively) and among populations ($F_{2,126} = 7.25$, $P = 1.05\times10^{-3}$ and $F_{2,103} = 12.32$, $P = 1.59\times10^{-5}$, respectively). Both resBWG and BWG_1% were greater at 18°C than at 24°C, while the AT fish had significantly lower resBWG and BWG_1% than WM fish ($P = 2.30\times10^{-2}$ and 6.73×10^{-3} with Tukey post-hoc test, respectively) and EM fish ($P = 1.02\times10^{-3}$ and $P = 2.79\times10^{-5}$ with Tukey post-hoc test, respectively). These traits were not significantly different between WM and EM fish ($P = 0.70$ and $P = 0.31$, respectively). There was no temperature by population interaction ($F_{2,124} = 0.58$, $P = 0.56$ and $F_{2,101} = 0.21$, $P = 0.81$, for resBWG and BWG_1%, respectively).

Regarding RMR and SMR, means for temperatures and populations are presented in Table 1, with RMR values in Fig. 1. Firstly, RMR was positively and strongly correlated with

SMR, whatever the temperature by population combination (r = 0.60-0.97). Thus, we chose to focus only on RMR results because SMR has very similar variations. Within each temperature by population combination, individual RMR was moderately variable with a CV (100*standard deviation/mean) between 9.8 and 14.8%. Moreover, RMR differed significantly by temperature, with fish reared at 18°C having lower RMR ($F_{1,116} = 130.89$, $P < 2.2\times10^{-16}$), but also by population ($F_{2,116} = 14.02$, $P = 3.52\times10^{-6}$), whereby AT fish had a significantly greater RMR than WM and EM fish ($P = 1.95\times10^{-4}$ and $P = 1.81\times10^{-4}$ respectively using Tukey post-hoc test). In contrast, the RMR of WM and EM fish was not significantly different (P = 0.94). Moreover, there was no interaction effect between temperature and population on RMR ($F_{2,114} = 1.67$, P = 0.19).

Correlation between Performance and RMR

As illustrated in Fig. 2, a link between RMR and resBWG as well as BWG_1% appears at temperature and populations levels. Indeed, fish at 18°C have a lower RMR and greater resBWG and BWG_1% than 24°C. Similarly, AT fish have a greater RMR and lower resBWG and BWG_1% than WM and EM fish.

At the individual level, the correlations between RMR and resBWG ranged from -0.33 to 0.39 among the various temperature by population combinations (Fig. 3), but none of them was significant (P > 0.05 in all cases). Similarly, the correlations between RMR and BWG_1% ranged from -0.43 to 0.15 among the various combinations (Fig. 4) and none of them was significant (P > 0.05 in all cases).

DISCUSSION

This study is the first to attempt to relate individual variation in FE to metabolic rate in a farmed fish, the European sea bass. The results revealed effects of temperature and population on FE that could be linked to differences in RMR. No such relationships were observed at an individual level.

Link between Feed Efficiency and RMR between Temperatures and among Populations

The initial hypothesis made was that the most efficient fish, for a given feeding rate (1% of MBW.day^{-1} in the present study), were those allocating the least energy to maintenance and activity, resulting in more available energy for growth. Present RMR results tend to valid this hypothesis between temperatures and among populations. Indeed, RMR differed in a consistent way with BWG_1%: fish at 18°C were more efficient for 1% of MBW.day^{-1} and had a lower RMR that at 24°C, AT fish were less efficient for 1% of MBW.day^{-1} and had a greater RMR than WM and EM fish.

Given that we estimated resBWG for fish fed *ad libitum*, it is perhaps surprising that it was also linked to RMR. It might have been suggested that fish with greater metabolic costs (i.e. RMR) would have compensated by increasing their *ad libitum* energy intake. However, our resBWG data suggest metabolic costs outweighed any potential compensation through an increased energy intake. This contrasts with Chinese crucian carp (*Carassius auratus*) where fish with greater RMR were less efficient under a restricted feeding rate but more efficient when fed at *ad libitum* (Zeng et al., 2017). This difference in results may be explained by the fact that fish were reared as a group by Zeng et al. (2017) but individually in the present study. Indeed, in the present study, *ad libitum* FI ranged from 0.53 to 0.73% and from 0.85 to 1.12% of BW.day^{-1} at 18°C and 24°C, respectively (Rodde at al., 2020). This is low compared to what could be expected in group rearing: around 1.1% and 1.7% of BW.day^{-1} at 18°C and 24°C, respectively, according to the model developed by Lanari et al. (2002) for European sea bass. It suggests that European sea bass do not achieve their full feed consumption potential during individual rearing.

The fact that RMR was greater in fish reared at 24°C than in those reared at 18°C is not surprising: oxygen consumption is known to increase with temperature in every fish species (meta-analysis by Clarke and Johnston, 1999), including European sea bass

(Claireaux and Lagardère, 1999). In contrast, it is very interesting that AT fish had a greater RMR than the two Mediterranean populations. This has, to our knowledge, never been reported before, although they are known to be genetically distinct (Duranton et al., 2018) and differ in other phenotypic traits such as growth, sex ratio, muscle fat or resistance to viral nervous necrosis (Guinand et al., 2017; Doan et al., 2017; Vandeputte at al., 2019). One event that may have provided a genetic basis for differences in metabolic rate among the populations is an ancient admixture between the Atlantic European sea bass and the closely related *Dicentrarchus punctatus* (Duranton et al., 2020). This led to the subsequent rapid fixation of some *D. punctatus* alleles in the Atlantic *D. labrax* and to the establishment of reproductive isolation barriers between Atlantic and Mediterranean populations (Duranton et al., 2020).

The phenotypic traits underlying such differences among populations in metabolic rate still need to be determined. It seems unlikely that variation is due to behavioural differences, because RMR was so strongly correlated with SMR, which indicates that animals were routinely very close to their basal metabolism. This can be explained by the fact that fish exhibited little swimming activity while in the individual respirometry chamber. Even if the experimental set-up avoided any disturbance from the outside, such little activity appears surprising. This may be due to the fact these fish had already experienced 123 days in isolated aquaria before being evaluated for RMR. Thus, the fish used here were probably more acclimated to isolation than usual, resulting in a low swimming activity. Other factors may be account for the RMR differences among populations. For instance, greater RMR might be associated to bigger sizes of metabolically expensive organs such as heart, liver or brain (Konarzewski and Książek, 2013), greater mitochondrial density (i.e. energy consumption per unit mass of tissue), greater activity of mitochondrial enzymes or lower ATP production efficiency (i.e. ATP produced per unit consumption of oxygen; Norin and Metcalfe, 2019).

Investigating these various hypotheses could provide a better understanding of the factors underlying RMR variation among populations.

Link between Feed Efficiency and RMR at Individual Level

Differences observed between temperatures and among populations revealed a consistent link between high FE performance and low RMR, whatever the feeding rate. In contrast, no correlation appeared at the individual level, no matter if the feeding rate was restricted (1% of MWB.day^{-1}) or not (*ad libitum*). Nevertheless, there is a need for further investigation before concluding that RMR is of no use to improve FE in a selective breeding program.

Firstly, only genetic correlations permit the conclusion whether a trait can be selected indirectly using another trait. At the individual level, a CV of 9.8 to 14.8% was found for RMR. Similarly, Killen et al. (2011) found a CV of 13% for European sea bass RMR, measured by the same respirometry method and then corrected for metabolic body weight. There is a need to determine whether this phenotypic variation in RMR has any heritable genetic component. However, setting up an experimental design to estimate genetic correlations is technically challenging. The number of fish phenotyped for both FE and RMR would need to be multiplied by at least four or five in comparison with the present study.

Moreover, fish BWG and FI performance were measured before RMR, and not simultaneously. The time lapse between these measurements was 12 weeks and fish had their weight multiplied by four, so their development stage was not similar, and this may explain why a correlation was not found. Metabolic rate estimation is known to have a moderate short term-repeatability in European sea bass (r = 0.48 for measurements separated by 20 minutes; Marras et al., 2010), but its longer term repeatability is, to our knowledge, unknown in this species. This is problematic because long-term repeatability of metabolic rate may be species-specific. For instance, it was reported as high (r = 0.68 for measurements separated by 17

386 weeks) in Atlantic salmon *Salmo salar* (McCarthy, 2000) but as very low (r = 0.093 for
387 measurements separated by 15 weeks) in brown trout *Salmo trutta* (Norin and Malte, 2011).
388 Similarly, the long-term repeatability of individual FE is completely unknown in the
389 European sea bass. Since it is not technically feasible to estimate individual FE and RMR
390 simultaneously, further investigation of both traits' long-term repeatability is needed.
391 It is also unknown whether the type of reserves, i.e. proteins or lipids, on which each
392 fish relies the most to produce its energy. Indeed, lipids provide twice as much energy as
393 proteins do for an equal weight. Thus, to ensure equal maintenance costs, fish degrading
394 lipids will consume a lower mass of reserves than fish degrading proteins. For instance,
395 McKenzie et al. (2014) reported that European sea bass relying on proteins rather than on
396 lipids to produce energy while fasting were losing more weight. Consequently, a link between
397 the main type of reserves used and individual FE may exist. In particular, AT fish muscle fat
398 content is greater than in the Mediterranean populations (Vandeputte et al., 2014; F. Allal,
399 personal communication, 2020). Thus, AT fish might use their lipid reserves less than
400 Mediterranean populations (and so they tend to accumulate them), degrading their protein
401 reserves instead. This could explain why AT fish are ultimately less efficient. This hypothesis
402 is supported by results reported in several species such as pig or rainbow trout showing the
403 most efficient animals had the lowest muscle fat content (Kamalam et al., 2012; Kause et al.,
404 2016; Knap and Kause, 2018).
405 Our results demonstrated variation among European sea bass populations regarding
406 oxygen consumption, in addition to the well-known effect of temperature on this trait.
407 Between temperatures and among populations, fish with a lower oxygen consumption were
408 more efficient. However, at the individual level, no significant correlation was found. Further
409 investigation is still required to fully understand the link between individual FE and oxygen
410 consumption in fish.

DISCLOSURES

The authors declare that they have no conflict of interest.

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556 ***Tables and Figures***

557 **Table 1.** Mean ± standard deviation (100*standard deviation/mean) of routine metabolic rate
558 (RMR), standard metabolic rate (SMR) and weight at respirometry. Results are presented for
559 Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at
560 18°C or 24°C. Within each combination of temperature by population, the correlation
561 between RMR and SMR is given with P-value, as well as the number of fish

562 **Fig. 1.** Routine metabolic rate (RMR) values observed for each combination of temperature
563 by population. Results are presented for Atlantic (AT), West Mediterranean (WM) and East
564 Mediterranean (EM) populations reared at 18°C or 24°C. In the box and whisker plots
565 presented, the box lower and upper limits are respectively the 0.25 and 0.75 quantiles of the
566 RMR data and the box is divided by the median of the values. The whiskers lower and upper
567 ends are respectively the lowest and greatest RMR values. Dots represent each fish RMR

568 **Fig. 2.** A) Residual body weight gain at *ad libitum* feeding rate as a function of routine
569 metabolic rate (RMR) among temperature by population combinations. B) Predicted body
570 weight gain as a function of RMR among temperature by population combinations. Predicted
571 body weight gain is expressed in % of metabolic body weight (MBW) and is corresponding a
572 level of feed intake set to 1% of $MBW.day^{-1}$. Results are presented for Atlantic (AT), West
573 Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C.
574 Horizontal and vertical bars associated to each point are corresponding to standard errors

575 **Fig. 3.** Individual residual body weight gain at *ad libitum* feeding rate as a function of
576 individual routine metabolic rate (RMR). Results are presented for Atlantic (AT), West
577 Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or 24°C. The
578 straight lines represent the linear regressions of individual residual body weight gain as a
579 function of individual RMR in each case

580 **Fig. 4.** Individual predicted body weight gain as a function of individual routine metabolic
581 rate (RMR). Predicted body weight gain is expressed in % of metabolic body weight (MBW)
582 and is corresponding a level of feed intake set to 1% of MBW.day^{-1}. Results are presented for
583 Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at
584 18°C or 24°C. The straight lines represent the linear regressions of individual predicted
585 weight gain as a function of individual RMR in each case
586

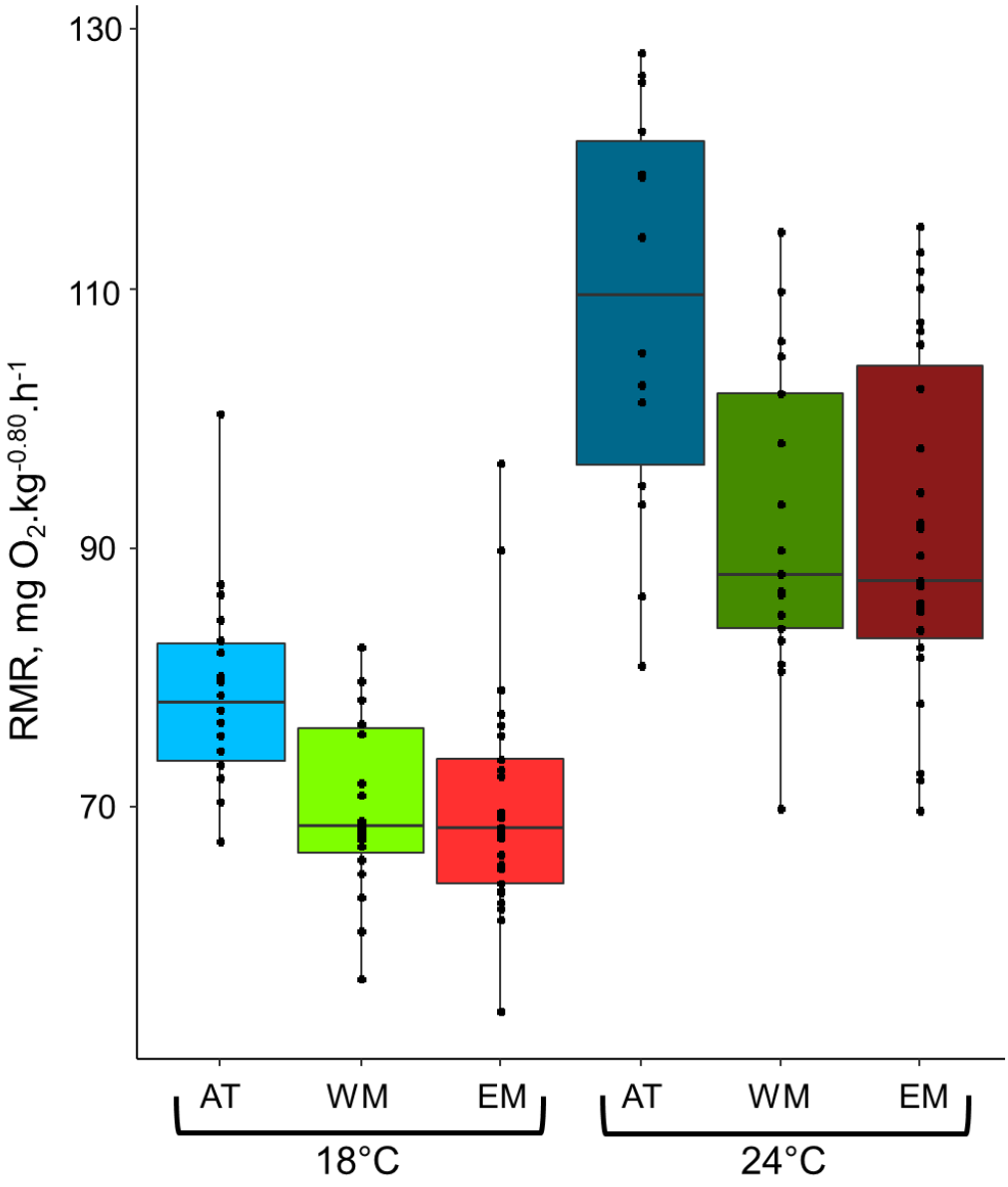
587 **Table 1**

588 Mean ± standard deviation (100*standard deviation/mean) of routine metabolic rate (RMR),
589 standard metabolic rate (SMR) and weight at respirometry. Results are presented for Atlantic
590 (AT), West Mediterranean (WM) and East Mediterranean (EM) populations reared at 18°C or
591 24°C. Within each combination of temperature by population, the correlation between RMR
592 and SMR is given with P-value, as well as the number of fish

	RMR, mg $O_2.kg^{-0.8}.h^{-1}$	**SMR, mg $O_2.kg^{-0.8}.h^{-1}$**	**Weight at respirometry, g**	**Correlation between RMR and SMR (P-value)**	**Number of fish**
Combinations					
AT x 18°C	78.7 ± 8.0 (10.2)	67.8 ± 4.7 (7.0)	107.6 ± 34.9 (32.5)	0.60 (P < 0.01)	18
WM x 18°C	70.1 ± 6.9 (9.8)	63.8 ± 5.8 (9.1)	80.0 ± 20.8 (25.9)	0.88 (P < 0.001)	19
EM x 18°C	70.3 ± 9.0 (12.9)	65.4 ± 7.2 (11.0)	107.2 ± 25.5 (23.8)	0.92 (P < 0.001)	25
AT x 24°C	108.5 ± 15.7 (14.5)	86.8 ± 13.9 (16.0)	142.2 ± 39.8 (28.0)	0.83 (P < 0.001)	14
WM x 24°C	91.9 ± 12.1 (13.2)	80.7 ± 10.4 (12.8)	125.5 ± 26.9 (21.4)	0.91 (P < 0.001)	17
EM x 24°C	91.3 ± 13.6 (14.8)	80.8 ± 13.1 (16.2)	161.3 ± 42.7 (26.5)	0.97 (P < 0.001)	27

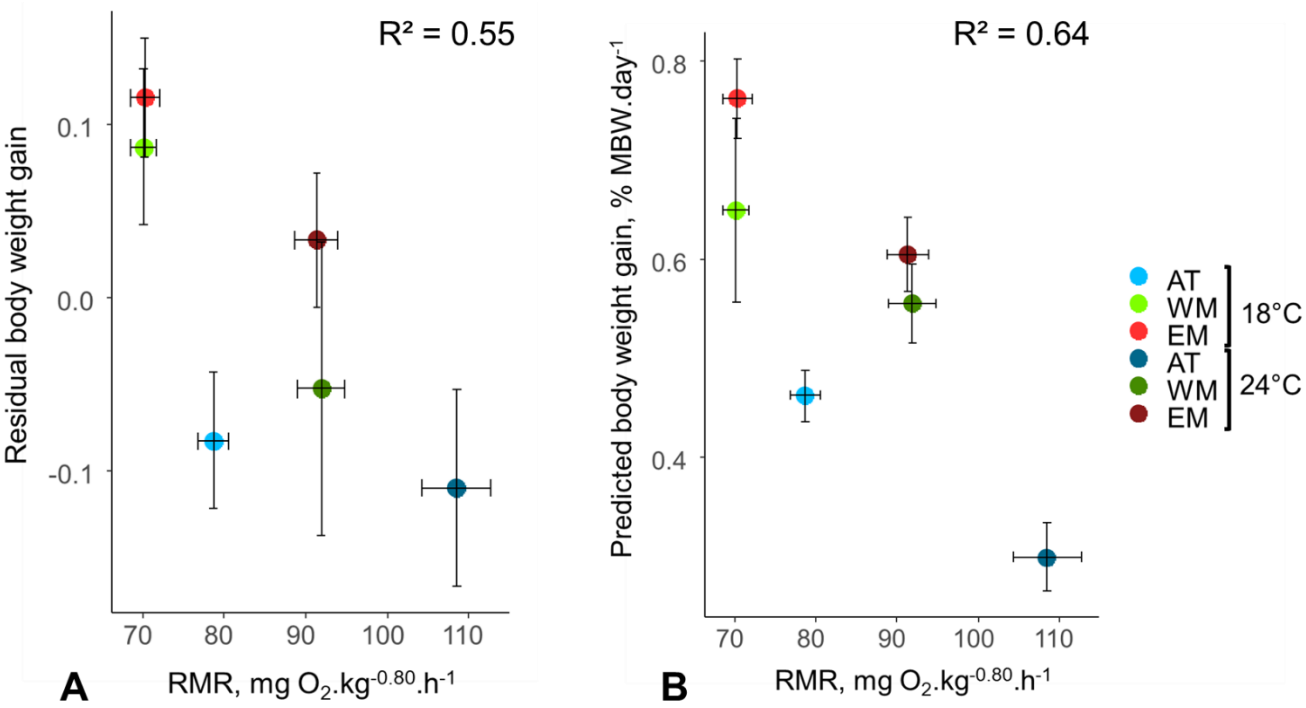
593

594



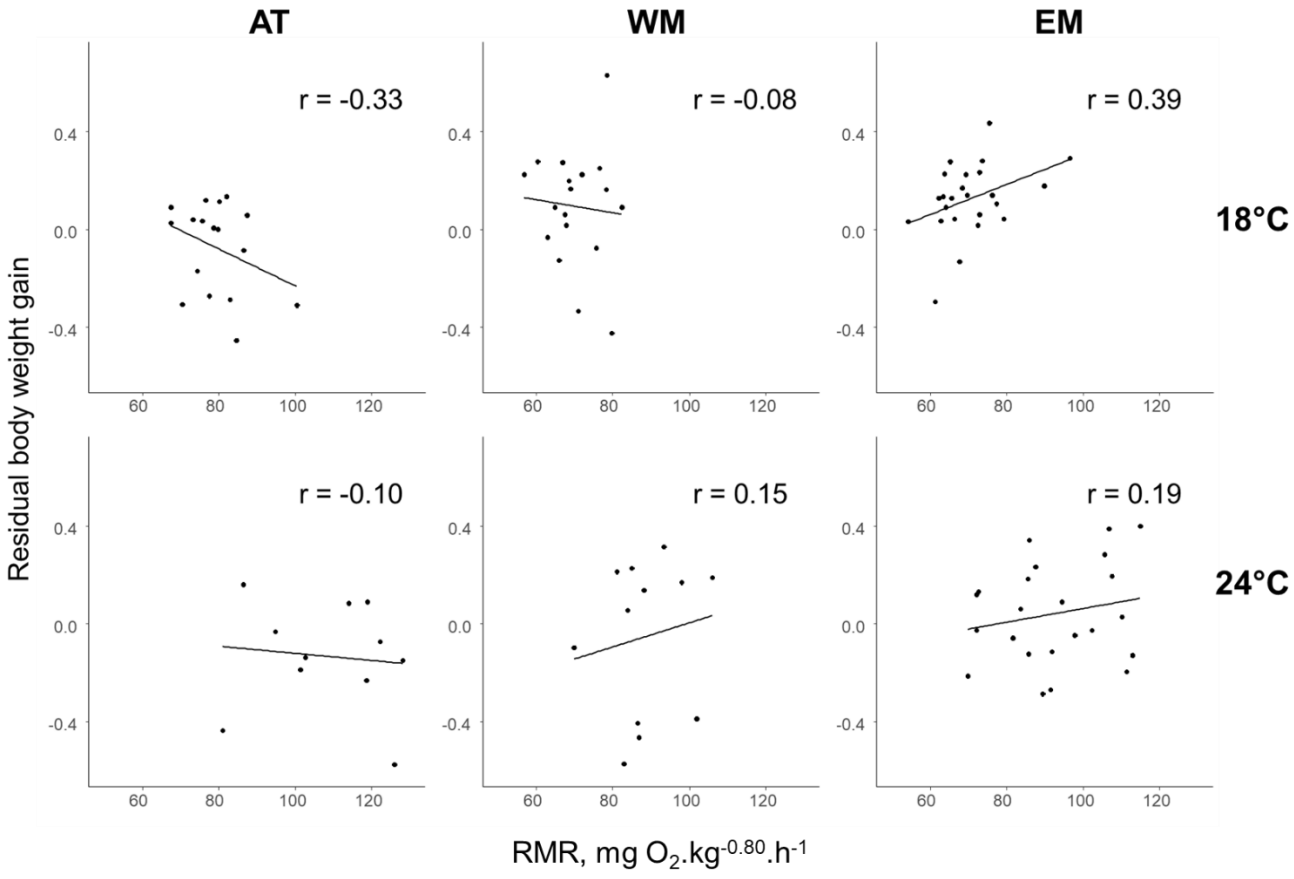
595

596 **Fig. 1.**


R² = 0.55
Residual body weight gain
0.1
0.0
-0.1
70
80
90
100
110
A
RMR, mg O2.kg-0.80.h-1
R² = 0.64
Predicted body weight gain, % MBW.day-1
0.8
0.6
0.4
B
AT
WM
EM
18°C
AT
WM
EM
24°C
RMR, mg O2.kg-0.80.h-1

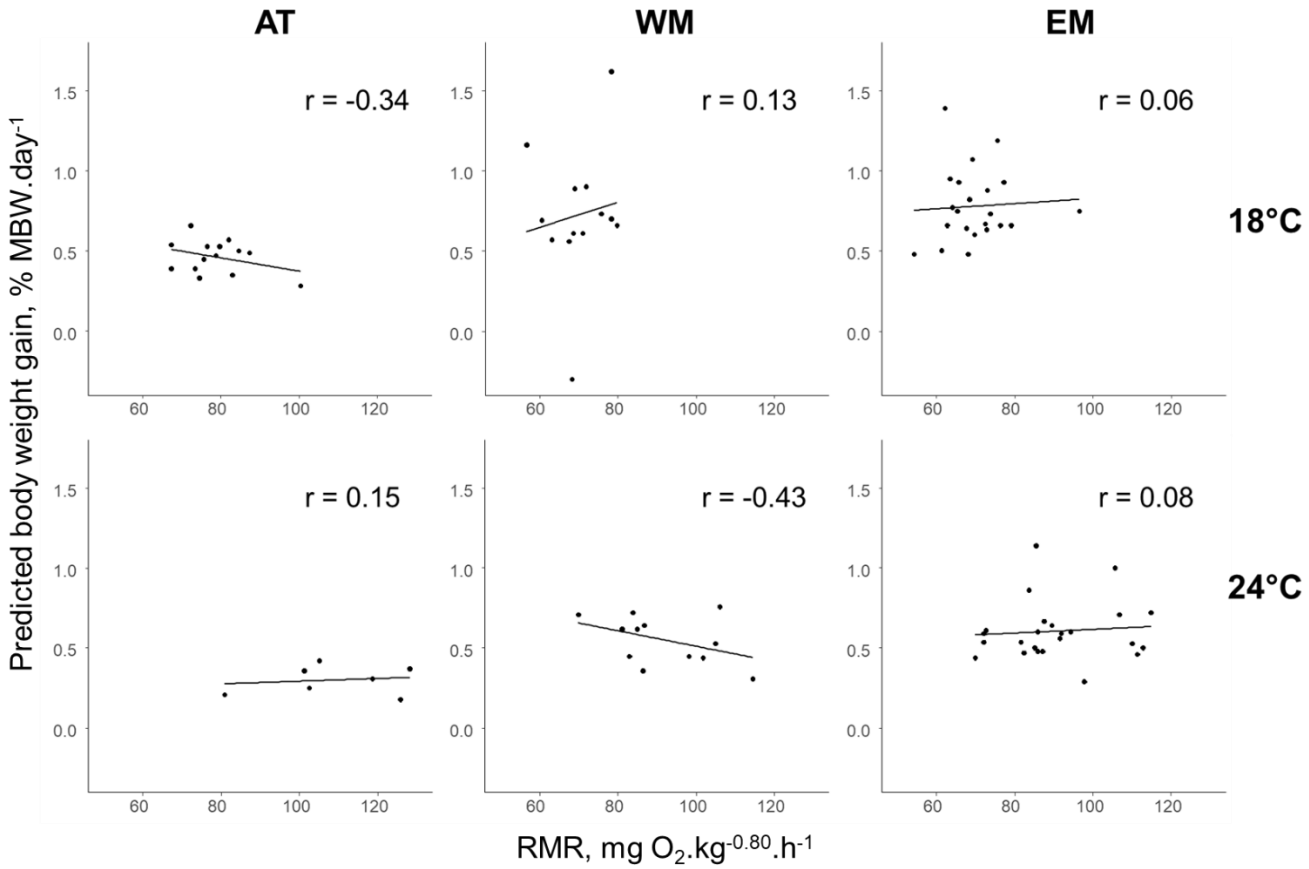
597

598 **Fig. 2.**


AT
WM
EM
r = -0.33
r = -0.08
r = 0.39
r = -0.10
r = 0.15
r = 0.19
18°C
24°C
Residual body weight gain
RMR, mg O2.kg-0.80.h-1

599

600 **Fig. 3.**



601

602 **Fig. 4.**

603